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Designing Spider Silk Genes for Materials Applications

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University of Wyoming
Laramie, WY

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project is designed to create new proteins based on naturally occurring spider silk proteins with the goal of controlling elasticity and tensile strength in fibers spun from the proteins. The new genes have been constructed in year 1, the proteins have been expressed and purified in this, year 2, and characterized to insure they are correct. We have now spun fibers from the produced silk proteins and started preliminary mechanical testing. The spinning process will need to be optimized but fibers have been produced with diameters as small as 8um and tensile strengths within 25% of the natural dragline silk fibers.

15. SUBJECT TERMS
spider silk, protein, fiber, mechanical testing

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Randolph V. Lewis
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Objectives: No changes

Status of Effort:

We have produced proteins and characterized them. One protein has been spun into a fiber with properties that approach those of the natural silk fibers. We are on track with the research with key accomplishments. The key goals for the next year are to optimize the spinning process for each of the new proteins, obtain mechanical testing data and compare that to the protein sequences.

Accomplishments:

We constructed twelve new spider silk genes that range from entirely elastic motifs to entirely strength motifs in year 1. Eight of them produced proteins of approximately 75 kdal in *E. coli*. The expression levels varied by at least a factor of five between the various constructs but all produce detectable protein. This period, year 2, we have scaled up the protein production to have enough protein to spin fibers and produce thin films for four of them and are continuing on the other four. The proteins have all been tested for the correct molecular weight via MALDI-TOF and for amino acid composition, which matches that expected.

We have started spinning fibers from the synthetic silk proteins. There was a several month delay in this process due to extraordinary difficulties in getting the spinning equipment shipped from Canada to here. Nexia donated the equipment to us and that seems to derail the U.S. Customs Service. So it took nearly four months and 10 hours of phone discussions with at least 8 different groups to get the equipment to UW. We have now spun fibers from all of the proteins (nine different ones) with varying degrees of success. Unfortunately there does not seem to be a common methodology that can be applied to all of the proteins. The common features are limited to extrusion into an organic alcohol and the need to conduct postspin draw to achieve the highest mechanical properties. Work is continuing on the best methods for fiber formation.

The very first fibers to be made ranged down to a diameter of 8 um (close to the natural *Nephila*) and had tensile strengths of about 25% of the natural fibers. We have subsequently generated fibers with diameters down to 5 um, tensile strengths to 50% of natural fibers and some with elongations of nearly 200%. These properties are likely to improve with postspin draw.

The new gene constructs and proteins represent an entirely new approach to understanding the basis for spider silk's strength and elasticity as well as the first systematic test of our ability to control both of these properties. With the ability to control the properties of the spider silk fibers and films they can be used for a much broader range of military and civilian applications.

Personnel supported:

Dr. Florence Teule
Shane Nelson, graduate student
Dagmara Motriuk-Smith, graduate student (received a PhD in May 2005 and is now on the faculty at UW, Casper)
Sherry Adrianos, undergraduate (non-traditional, female, first generation college), is now a graduate student in our department
Josh Duncan, undergraduate (now in medical school at the Uniformed Services Medical University)
Alyssa Cooper, undergraduate with joint degree in Engineering and Molecular Biology
William Furin, undergraduate and now a Research Technician

Publications:

(2005) Brooks, A.E., Creager, M. and Lewis, R.V., "Altering the Mechanics of Spider Silk Through Methanol Post-spin Draw", Biomedical Sciences Instrumentation:Vol. 41, pg: 1-6.
(2005) Amanda E. Brooks,* Holly B. Steinkraus, Shane R. Nelson, and Randolph V. Lewis, An Investigation of the Divergence of Major Ampullate Silk Fibers from *Nephila clavipes* and *Argiope aurantia*, Biomacromolecules 6: *3095-3099*
(2005) C. Wong Po Foo, E. Bini, J. Hensman, D.P. Knight, R.V. Lewis and D.L. Kaplan, Role of pH and charge on silk protein assembly in insects and spiders, Applied Physics A: Materials Science & Processing 82: 223 - 233

Interactions/Transitions:

a. Meetings: Air Force meeting in San Diego, Seminars at Iowa State, Utah State, b. Collaborations: Ron Eby, Wright State U. (Air Force Grantee)
c. Transitions: None

Patent disclosures: No new ones.

Honors and Awards: University of Wyoming Distinguished Faculty for 2005.